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(54) **POLYSACCHARIDE GEL COMPOSITION**

GELZUSAMMENSETZUNG AUF BASIS VON POLYSACCHARIDEN

COMPOSITION DE GEL A BASE DE POLYSACCHARIDE

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Description

TECHNICAL FIELD

[0001] The present invention relates to the field of biocompatible polysaccharide gel compositions, and more specifically to a novel process for cross-linking such compositions, a new gel structure thereby being obtained. The new structure imparts improved properties to the previously known gel compositions as well as enables new uses of said compositions, both as such and containing active ingredients.

BACKGROUND OF THE INVENTION

[0002] Water-binding gels are widely used in the biomedical field. They are generally prepared by chemical cross-linking of polymers to infinite networks. When using biocompatible polymers generally a low degree of cross-linking has to be utilized to maintain said biocompatibility. However, often a more dense gel is required to have a proper effect of the active ingredients utilized, and in such a case the biocompatibility will often go lost.

[0003] Another valuable property of water-binding gels, or hydrogels, is that peptides and larger biologically active substances can be enclosed therein to the formation of a sustained release composition. However, practical problems have been involved in accomplishing a sufficient maintenance time of the active ingredient, since generally the active ingredient is released at the same rate with which it was dissolved or enclosed in the composition referred to. Furthermore, if such a gel were densified in an attempt to maintain the active ingredient for a longer time, it would rapidly swell in an animal tissue where there is a free access of water.

[0004] One of the most widely used biocompatible polymers for medical use is hyaluronic acid. As it is present in identical composition in each living organism, it gives a minimum of reactions and allows for advanced medical uses. As a consequence thereof it has been the subject of many modification attempts. Thus, it has been cross-linked with agents such as aldehydes, epoxides, polyaziridyl compounds and divinylsulfone (Laurent et al, Acta Chem. Scand 18 (1964) No 1, p. 274; EP 0 161 887B1; EP 0 265 116A2; and US 4,716,154).

[0005] In WO 87/07898 there is disclosed a reaction of a polysaccharide with a polyfunctional epoxide, removal of excess of said epoxide and finally drying operation to cross-link said polysaccharide into a film, powdered material or similar dry product. However, there is no suggestion therein to dilute the activated polysaccharide and then reconcentrate the same to the desired density or consistency which is then substantially permanent.

[0006] US 5,128,326 discloses a number of modified hyaluronic acids for use as depot pharmaceuticals. The disclosed methods of "charging" the gel preparations are all based on a diffusion of the active ingredient into the gel and then a release thereof with the same diffusion constant. Contrary thereto the present invention involves a dissolution of the active ingredient followed by a densification or concentration of the gel composition until no or a very minor diffusion of said active ingredient takes place.

[0007] US 5,399,351 discloses mixtures of gel and polymeric solutions, said solutions being utilized to improve the rheological properties of the gel. However, also in this case reversibly compressed gels are disclosed, as can be gathered from e.g. col. 6, lines 53-58.

SUMMARY OF THE INVENTION

[0008] According to the present invention it has unexpectedly been found that polysaccharide gel compositions having a novel structure and thereby new, outstanding properties can be obtained by using a new technique for the cross-linking thereof. Said new cross-linking technique enables a versatile control of the structure and properties of the manufactured polysaccharide gel composition, which in turn makes it possible to tailor the final composition for the inteded purposes.

[0009] More specifically, one object of the present invention is to provide a process for preparing a cross-linked polysaccharide gel composition, the biocompatibility of which can be retained in spite of a high degree of cross-linking or polymerisation.

[0010] Another object of the invention is to provide a polysaccharide gel composition with viscoelastic properties in spite of being cross-linked to a substantial degree.

[0011] Yet another object of the invention is to provide a polysaccharide gel composition which is more or less irreversably densified or concentrated, i.e. which does not swell substantially or only to a limited degree when contacted with water.

[0012] Still another object of the invention is to provide a polysaccharide gel composition enclosing a biologically active substance for use as a sustained release composition or a depot composition.

[0013] Another object of the invention is to provide polysaccharide gel compositions containing a variety of biologically active substances for uses as medical or prophylactic compositions for different purposes.

[0014] Yet another object of the invention is to provide uses of the compositions referred to for the manufacture of medical or prophylactic compositions as well as for administration to mammals, especially humans.

[0015] Yet another object of the invention is to provide a partially cross-linked activated polysaccharide gel composition as obtained as an intermediate in the above-mentioned process according to the invention, which intermediate can be finally cross-linked in situ at any desired site.

[0016] These and further objects of the invention will become apparent by the more detailed description thereof presented below.

DETAILED DESCRIPTION OF THE INVENTION

[0017] According to one aspect of the present invention a process for preparing a cross-linked biocompatible polysaccharide gel composition is thus provided, which process comprises:

forming an aqueous solution of a water soluble, cross-linkable polysaccharide;

initiating a cross-linking of said polysaccharide in the presence of a polyfunctional cross-linking agent therefor;

sterically hindering the cross-linking reaction from being terminating before gelation occurs, an activated polysaccharide thereby being obtained; and

reintroducing sterically unhindered conditions for said activated polysaccharide so as to continue the cross-linking thereof up to a viscoleastic gel.

[0018] In other words the new process according to the present invention involves a cross-linking of a watersoluble, cross-linkable polysaccharide in at least two steps or stages, where the cross-linking reaction is discontinued before the gelation is initiated, said discontinuance being accomplished by sterically hindering said cross-linking reaction. The cross-linking reaction is then continued in a second step by reintroducing sterically unhindered conditions.

[0019] Thus, firstly is has unexpectedly been found that by said sterical hindrance an activated polysaccharide is obtained, the cross-linking or polymerization of which can be continued merely by reintroducing sterically unhindered conditions therefor. Secondly, it has also unexpectedly been found that the polysaccharide gel composition obtained thereby does not form the compact, dense structure which would have been obtained if performing the corresponding cross-linking reaction in one single step to a fully cross-linked gel but rather a viscoleastic gel. Furthermore, as was mentioned above, the new gel structure obtained by the present invention represents a substantially irreversible gel structure which does not swell to any appreciable extent in contact with water or any other aqueous medium. Generally this means that said reswelling is less than 10% by volume based on the volume as obtained from the process claimed.

[0020] Although the invention is not bound by any theory it may be that the new structure obtained by the present invention is a combination of cross-linking between existing polymer chains and an elongation of existing chains rather than a very dense network giving a very rigid structure. What may suggest such a mechanism is the fact that a viscoelastic product is obtainable by the invention.

[0021] As used herein the term "sterically hindering the cross-linking reaction" should be interpreted in a broad sense, i.e. it need not necessarily be a complete hinderance but in many cases rather a partial hindrance of the reaction referred to. That is, what is important is that the rate of cross-linking is substantially reduced to enable the final cross-linking reaction to take place with new reaction sites involved.

[0022] Similarly, the term "reintroducing sterically unhindered conditions" should also be interpreted broadly, which generally means that said sterically unhindered conditions need not necessarily be exactly the same sterical conditions as were used when initiating the cross-linking reaction. Thus, what is generally of importance is that said sterically unhindered conditions enable more rapid reactions to take place than said sterically hindered conditions.

[0023] The sterical hindrance of the cross-linking reaction should be obtainable in different ways, but a preferred embodiment of the invention in this respect is represented by the case where the sterical hindrance comprises diluting the aqueous medium in which the cross-linking reaction is performed, to accomplish a lower concentration of the polysaccharide in said medium.

[0024] To reintroduce sterically unhindered conditions should also be possible in different ways, but a preferred embodiment in this respect is the case which comprises evaporating the aqueous medium in which the cross-linking reaction is performed, to accomplish a higher concentration of the polysaccharide in said medium. Another preferred embodiment in this respect is represented by the case comprising dialysing the aqueous medium in which the cross-linking reaction is performed.

[0025] According to a preferred embodiment of the invention the sterical hindrance of the cross-linking reaction is accomplished before the cross-linking agent has been consumed. This in turn generally also means that the reintro-

duction of sterically unhindered conditions is initiated in the presence of said non-consumed cross-linking agent.

[0026] The sterical hindrance of the cross-linking reaction can generally be started or performed in the range of 50-90% of the total gelation time used in the process according to the invention, consideration also being taken to suitable elasticity or consistency for the intended use of the composition.

[0027] The inventive idea should be applicable to any biocompatible polysaccharide that is cross-linkable and soluble in an aqueous medium. Thus, the term "water soluble" should be interpreted in a broad sense, pure water not necessarily being necessary. That is, aqueous solution means any solution wherein water is the major component. A preferred sub-group of polysaccharides in connection with the invention is, however, a glucose amine glucan, of which hyaluronic acid is a specially interesting example.

[0028] The cross-linking agent to be used in connection with the invention is any previously known cross-linking agent useful in connection with polysaccharides, consideration being taken to ensure that the biocompatibility prerequisites are fulfilled. Preferably, however, the cross-linking agent is selected from the group consisting of aldehydes, epoxides, polyaziridyl compounds, glycidyl ethers and dividylsulfones. Of these glycidyl ethers represent an especially preferred group, of which 1,4-butandiol digylcidylether can be referred to as a preferred example. In this connection it should also be mentioned that "polyfunctional" includes difunctional.

[0029] The initial cross-linking reaction in the presence of a polyfunctional cross-linking agent can be performed at varying pH values, primarily depending on whether ether or ester reactions should be promoted. Preferably this means that said cross-linking reaction is performed at an alkaline pH, especially above pH 9, e.g. in the range of pH 9-12, when promoting ether formations. When promoting ester formations said cross-linking reaction is preferably performed at an acidic pH, especially at pH 2-6.

[0030] One interesting aspect of the invention is represented by the case where the prepared cross-linked polysaccharide gel composition is utilized as such as the invention enables the manufacture of a viscoelastic composition. Such a viscoelastic composition is for instance useful in eye surgery, as a synovial fluid substitute and as eyedrops, and as has been referred to above the present invention makes it possible to tailor the viscoelastic properties for such uses. Thus, by utilizing the sterical technology according to the present invention it is possible to obtain chain extensions, chain branchings and cross-links in a more controlled way that by the previously used techniques with more or less randomized coupling sites.

[0031] Furthermore, through the fact that the gels obtained in accordance with the invention do not retain their original volume in the presence of an aqueous medium, the new products do not cause any interfering or negative volume effects in these or other medical uses.

[0032] In accordance with the present invention it is also possible to include within the polysaccharide gel composition any biologically active substance for which a polysaccharide gel carrier is desired or accepted. In this context the dilution-concentration technique used in the process claimed enables the enclosure of said biologically active substance before subjecting the polysaccharide to sterically unhindered conditions. That is, while sterically unhindered conditions generally means a concentrating operation, such an operation means that the biologically active substance will be present in a phase that is more compacted than when said substance was included in said carrier. In other words the biologically active substance can be retained much longer as compared to previously known gel cross-linking reactions. Thereby a better sustained release profile for the active substance is obtainable.

[0033] In connection with the incorporation of the biologically active substance into the composition an adjustment of the conditions to physiological pH and salt conditions is preferably performed to have a preparation ready for medical use. Such a physiological adjustment is preferred also as concerns the reaction conditions as the second step of the process has been found to proceed well under suchg conditions.

[0034] The invention should not be limited in any respect as to the biologically active substance as compared to the use of said substance in prior cases. In other words the condition to be treated should be decisive for the specific substance to be selected.

[0035] However, interesting substances in connection with the invention can be selected from the group consisting of hormones, cytokines, vaccines, cells and tissue augmenting substances. Thus, the unique combination of properties of the new gel composition according to the present invention makes it extremely advantageous in connection with these substances, i.e. primarily thanks to outstanding depot or sustained release properties and non-swelling properties.

[0036] One interesting group of biologically active substances thus is tissue augmenting substances as a polysaccharide gel is an advantageous carrier therefor. Further details concerning such products can be found in WO94/21299. More specifically, a preferred tissue augmenting substance comprises a polymer selected from collagen, starch, dextranomer, polylactide and compolymers thereof, and poly-β-hydroxibutyrate and copolymers thereof.

[0037] In connection with hormones erytropoeitin and calcitonin are especially preferred.

[0038] The process according to the present invention also enables the incorporation of the biologically active substance by chemical reaction with the polysaccharide gel structure, or the cross-linking agent therefor, provided that said active substance contains functional groups reactive therewith. Unique properties or combinations of properties

can thereby be obtained as in such a case for instance the release rate of the active ingredient will be decided by the degredation or decomposition of the polymer network rather than by the dissolution or migration rate for the substance referred to from the gel network.

[0039] A modification of last-mentioned technique in accordance with the invention means that the functional groups of the active substance may have been prereacted with a cross-linking agent for the polysaccharide. Preferably the same cross-linking agent is used as is used in the cross-linking of the polysaccharide.

[0040] Since the process of the present invention provides a new polysaccharide gel composition or structure, another aspect of the invention is represented by the novel polysaccharide gel composition prepared. In this respect the scope of protection encompasses not only the polysaccharide gel composition whenever prepared by said process but also any polysaccharide gel composition which is obtainable by a similar technique.

[0041] Expressed in another way the present invention also provides a cross-linked biocompatible polysaccharide gel composition, which is obtainable by cross-linking of a cross-linkable polysaccharide with a polyfunctional cross-linking agent therefor in two steps, the first cross-linking step being terminating before gelation occurs, by a sterical hindrance of the cross-linking reaction, and the second cross-linking step being initiated by reintroducing sterically unhindered conditions for said cross-linking reaction to continue the same up to a viscoelastic gel.

[0042] All those features which have been presented as preferred or interesting features in connection with the claimed process are applicable also to said polysaccharide gel composition per se and need not be repeated once more.

[0043] Still another aspect of the invention is represented by the case where an intermediate product is obtained by postponing the final step of the cross-linking reaction with sterically unhindered conditions to a later stage or site, for instance at the ultimate use of the composition. Thus, it has been found that the intermediate product obtained after the sterical hindrance of the cross-linking reaction possesses such a stability that the termination of the cross-linking reaction can be performed at a later stage.

[0044] The invention also relates to the composition defined above for use a medical or prophylactic composition.

[0045] Another aspect of the invention is the use of said composition for the manufacture of a medical or prophylactic composition for any of the above-mentioned specific medical or therapeutical purposes, tissue augmentation and hormone treatment of a mammal, especially a human being, being preferred applications.

EXAMPLES

[0046] The invention will now be exemplified by the following non-limiting examples.

Example 1

Activation of the polymer.

a. Under alkaline conditions

[0047] Polysaccharide in the form of 10 g of hyaluronic acid prepared by fermentation of Streptococcus were dissolved in 100 ml of 1% NaOH pH >9. Cross-linking agent in the form of 1,4-butandiol diglycidylether was added to a concentration of 0,2%. The solution was incubated at 40°C for 4 hours.

b. Under acidic conditions

[0048] The experiment was performed as in Ia but at an acidic pH of about 2-6 by the addition of 1% of acetic acid to the solution instead of NaOH according to 1a.

Example 2

Preparation of a viscoelastic gel.

[0049] The incubates according to Ia and Ib were diluted to a volume which was twice the volume finally desired or about 0,5-1% and were neutralised. The gel was then rotary evaporated to a viscoelastic gel.

Example 3

Preparation of a gel containing dextranomer particles.

[0050] The incubates according to 1a and 1b were diluted to a strength of 1% and 20 g dry dextranomer particles

(Sephadex®25, Pharmacia) were mixed with the solution, the particles being enclosed by the cross-linking of hyaluronic acid polymer in a few minutes as a consequence of the concentration of hyaluronic acid which is accomplished by an absorption of water by the dextranomer beads.

[0051] The viscoelastic gels obtained were stable, autoclavable and injectable by means of thin hypodermic needles.

Example 4

Preparation of a gel for use as a depot medicine containing erytropoeitin (EPO).

[0052] The incubate obtained in Example Ia was diluted to a strength of 1% and the pH was adjusted by the addition of a citrate buffert according to the instructions from the manufacturer (Ortho Biotech Inc., Raritan USA) for a good stability in aqueous solution. 5 x 10^6 IU of EPO were added under stirring. After evaporating the solution to 1/4 of the volume the polymer had been cross-linked to a depot composition and an amount of 20 000 IU of EPO/ml was recovered.

Example 5

Preparation of a gel for use as a depot preparation containing calcitonin.

[0053] Calcitonin from salmon 100 IU/ml (Miacalcic® Sandoz) were admixed with 2% of polymer solution manufactured in accordance with Example Ib and the solution was concentrated to 5% (250 IU/ml) by rotary evaporation. A horse with chronic claudication in the right front leg was treated with an injection of 2 ml s.c. per week during two weeks. In the six weeks following thereafter said horse was free from pains. The serum calcium was lowered with 12% only.

Example 6

Preparation of a gel containing heparin to be released in a sustained way.

[0054] In a diluted activated polymer according to Example 4 heparin was dissolved in an amount of 5% of the polymer. The mixture obtained was equilibrated for 1 hour, whereupon it was evaporated to 1/4 of the volume. A coagulation inhibiting release thereof was noted during 16 days of incubation in physiological saline.

Example 7

Preparation of a gel with covalently bonded heparin in a sterically controlled position.

[0055] Activated polymer according to Example 1 was precipitated in methanol under vigorous stirring. The fine-threaded precipitation obtained was dried during the night. Heparin was activated in accordance with example 1. After said incubation (4 hours at 40°C) the polymer precipitation was mixed with the activated heparin solution. The mixture was incubated during the night and the following day the gel solution was neutralised, particulated and washed from reactant residues.

[0056] The gel formed was able to bind growth factor, inter alia basic Fibroblast Growth Factor (bFGF), but did not show any inhibition of the coagulation of whole blood.

Example 8

Preparation of a gel containing positively charged groups of chitosan.

[0057] Incubation of a mixture of 7,5 g of hyaluronic acid polymer and 2,5 g of chitosan (See Cure® Protan) was performed in accordance with example 1. After a dissolution and a neutralisation a copolymerized viscoelastic solution was obtained. Said solution possessed healing promoting properties after having been applied to a sore slow in healing.

Example 9

Preparation of a gel which has been sterically coupled.

[0058] 7,5 g of hyaluronic acid were activated in accordance with Example Ia. In the same way 2,5 g of dextran were activated. The hyaluronic acid was precipitated in methanol, the precipitation then being mixed with 500 ml of a diluted

activated 0,5% dextran solution. After stirring and adjustment of pH and salt concentration a viscoelastic solution was obtained. 5 ml of said solution was infused in an Achilles tendon sheath which repeatedly showed inflammation in the form of soreness and "creaking". After four weeks said Achilles tendon problems had disappeared.

Example 10

Preparation of a gel for use as a medicinal depot containing GMCSF.

[0059] The product was prepared in accordance with Example 5 but instead of calcitonin there was added Granulocyte macrophage - colony stimulating factor, GMCSF (Leucomax®) 1 mg/g polymer.

Example 11

Preparation of gel containing killed virus type Influenza A2.

[0060] The preparation was performed as in Example 4 but instead of EPO 40 960 HAU killed influenza horse virus per 100 ml of diluted active 1% polymer solution were added. After contraction 4x the preparation contained 1 600 HAU per ml. By a vaccination of more than 100 horses in connection with an epidemic influenza the preparation was found to be highly effective as to protection against infection, which protection was maintained for a long time (more than 6 months).

Example 12

Preparation of a fresh gel containing a living cell suspension.

[0061] A 5 ml fibroblast culture was mixed with 100 ml of a neutralized solution according to example Ia. The mixture was oxygenated and dried to half the volume. A viscoelastic solution containing living cells was obtained.

Example 13

Preparation of a dense micronised gel containing small peptides.

[0062] To an activated neutralized gel according to Example Ia there was added 5 mg of a peptide having 12 amino acids. The gel was evaporated during stirring to 10% and was suspended in mineral oil. After addition of methanol the dry gel particles were filtered off and washed clean from oil residues.

Example 14

Preparation of a gel containing the dense micronised gel with small peptides according to Example 13.

[0063] To a 1% solution of neutralized polymer activated according to Example Ia microspheres from Example 13 were added. The gel was then evaporated to half its volume. A homogenous injectable and stable gel containing finely dispersed microspheres was formed.

Example 15

Preparation of a gel containing spherical polymethylmethacrylate (PMMA) beads having a size of 40 - 120 µm.

[0064] To 5 g of a polymer diluted to 1% and neutralized and activated according to Example Ia 100 mg of spheres of polymethylmethacrylate (PMMA) were added. Evaporation to 3% polymeric gel gave a stable injectable viscoelastic gel.

Example 16

Preparation of a gel containing PMMA fragments of 500 nm to which hydrophobic antigen has been added.

[0065] Haemagglutinin antigen prepared from A2 virus according to Example 11 was absorbed by hydrophobic interaction on 500 nm PMMA particles. Said particles were added to 1% solution according to Example 15 and a reduction

to half the volume was accomplished. A stable homogenous viscoelastic gel was formed which was useful as a vaccine having a high adjuvant effect.

Example 17

A comparison between the degree of reswelling at free availability of water between conventionally prepared gels and gels prepared according to the present invention.

[0066] Hyaluronic acid gels prepared according to Laurent et al 1963 and according to Examples 1 and 2 above were dried to half their swelling volumes. Then they were reintroduced into their original solutions. The previously known gels swelled to their original volume while the gel compositions according to the present Examples 1 and 2 swelled marginally only (10%).

Example 18

Comparison between biological activity of EPO copolymerized with hyaluronic acid to a gel and the gel according to Example 1 into which EPO had been enclosed by a concentration of said gel.

[0067] Four patients under treatment with Eprex® (CILAG) for their anaemia caused by chronic uraemia were treated for two months with a dose each month according to the following regimen:

Patient no.	1	2	3	4
Dose IU	60 000	70 000	70 000	50 000
Month 1	Directly gelled depot,	Directly gelled depot,	Control	Control
Month 2	Control	Control	Concentrated	Concentrated

Directly gelled depot: Epoxide cross-linking under mild conditions according to Example 11 in the presence of EPO.

Control: EPO dissolved in 4% hyaluronic acid MW about $6x10^6$ from cock's comb prepared according to US 4 141 973 (Healon® Pharmacia).

Concentrated: EPO enclosed within activated gel which was gelled through concentration.

[0068] The dose was selected as the total dose per month which was normally required by the patient to maintain the haemoglobin level. The serum level of EPO was analysed at regular intervals by means of an immunochemical method.

Results

[0069] A common method of expressing the functionary effect of depot preparations is to calculate the curve area (units of EPO x days). This study also gives the bioavailability in the form of haemoglobin level in blood as 0 = retained, + = increased and - = reduced.

Table

Patient no./month	1/1	1/2	2/1	2/2	3/1	3/2	4/1	4/2
Area under the curve	41	424	57	534	224	952	567	656
Haemoglobin control	-	+	-	+	0	+	+	+

Conclusion

[0070] An enclosure of EPO in a contracted depot gives the highest possible release during the analysis. Attempts to perform the gelling reaction in the presence of EPO destroyed the hormones such that a very low release could be registered.

Claims

1. A process for preparing a cross-linked biocompatible polysaccharide gel composition, which comprises:

   forming an aqueous solution of a water soluble, cross-linkable polysaccharide;

   initiating a cross-linking of said polysaccharide in the presence of a polyfunctional cross-linking agent therefor;

   sterically hindering the cross-linking reaction from being terminated before gelation occurs, an activated polysaccharide thereby being obtained; and

   reintroducing sterically unhindered conditions for said activated polysaccharide so as to terminate the cross-linking thereof up to a viscoelastic gel.

2. A process according to claim 1, wherein the polysaccharide is selected from the group consisting of glucose amine glucans.

3. A process according to claim 2, wherein said glucose amine glucan comprises hyaluronic acid.

4. A process according to any one of the preceding claims, wherein the cross-linking agent is selected from the group consisting of epoxides, polyaziridyl compounds and glycidyl ethers.

5. A process according to claim 4, wherein said glycidyl ether comprises 1,4-butanediol diglycidylether.

6. A process according to any one of the preceding claims, wherein said sterically hindering of the cross-linking reaction comprises diluting the aqueous medium in which the cross-linking reaction is performed, to accomplish a lower concentration of the polysaccharide in said medium.

7. A process according to any one of the preceding claims, wherein said reintroduction of sterically unhindered conditions comprises evaporating the aqueous medium in which the cross-linking reaction is performed, to accomplish a higher concentration of the polysaccharide in said medium.

8. A process according to any one of claims 1-6, wherein said reintroduction of sterically unhindered conditions comprises dialysing the aqueous medium in which the cross-linking reaction is performed.

9. A process according to any one of the preceding claims, wherein the initial cross-linking reaction in the presence of a polyfunctional cross-linking agent is performed at an alkaline pH, ether cross-linking reactions thereby being promoted.

10. A process according to claim 9, wherein said alkaline pH is above pH 9.

11. A process according to any one of claims 1-10, wherein the initial cross-linking reaction in the presence of a polyfunctional cross-linking agent is performed at an acidic pH, ester cross-linking reactions thereby being promoted.

12. A process according to claim 11, wherein said acidic pH is pH 2-6.

13. A process according to any one of the preceding claims, wherein said sterical hindrance of the cross-linking reaction is accomplished before said cross-linking agent has been consumed.

14. A process according to any one of the preceding claims, wherein a biologically active substance is enclosed within the cross-linked polysaccharide gel composition during the preparation thereof.

15. A process according to claim 14, wherein said biologically active substance is enclosed at physiological pH and salt concentration conditions.

16. A process according to any one of claims 14 and 15, wherein said active substance is enclosed within the gel composition by dissolving or dispersing the same in said activated polysaccharide before subjecting last-mentioned

polysaccharide to sterically unhindered conditions.

17. A process according to any one of claims 14-16, wherein said biologically active substance is selected from the group consisting of hormones, cytokines, vaccines, cells and tissue augmenting substances.

18. A process according to claim 17, wherein said tissue augmenting substance comprises a polymer selected from collagen, starch, dextranomer, polylactide and copolymers thereof, and poly-β-hydroxybutyrate and copolymers thereof.

19. A process according to claim 17, wherein said hormone is selected from the group consisting of erytropoeitin and calcitonin.

20. A process according to any one of claims 14-19, wherein said biologically active substance contains functional groups reactive with the polysaccharide and is enclosed within the gel structure by chemical reaction therewith.

21. A process according to claim 20, wherein said biologically active substance containing functional groups has been prereacted with a cross-linking agent for said polysaccharide.

22. A process according to claim 21, wherein said cross-linking agent is the same cross-linking agent as is used in the cross-linking of the polysaccharide.

23. A cross-linked biocompatible polysacharide gel composition preparable by a process as claimed in any one of claims 1-22.

24. A partially cross-linked biocompatible activated polysaccharide gel composition as obtained by a process as claimed in any one of claims 1-22 before continuing the cross-linking of the activated polysaccharide by reintroducing said sterically unhindered conditions for the cross-linking reaction.

25. A composition according to claim 23 for use as a medical or prophylactic composition.

26. A composition according to claim 25, which is adapted as a depot preparation.

27. Use of a composition according to claim 23 for the manufacture of a medical or prophylactic composition for tissue augmentation of a mammal, especially a human being.

28. Use according to claim 27 for the manufacture of a medical or prophylactic depot composition, especially for hormone treatment of a mammal, especially a human being.

Patentansprüche

1. Verfahren zur Herstellung einer vernetzten biokompatiblen Polysaccharidgelzusammensetzung, das umfaßt:

   Bilden einer wäßrigen Lösung eines wasserlöslichen, vernetzbaren Polysaccharids; Auslösen einer Vernetzung des Polysaccharids in Gegenwart eines polyfunktionellen vernetzenden Mittels;
   Sterisches Hindern der Vernetzungsreaktion bevor sie beendet ist und Gelbildung erfolgt, wodurch ein aktiviertes Polysaccharid erhalten wird;
   und Wiedereinführen der sterisch ungehinderten Zustände für das aktivierte Polysaccharid, um so die Vernetzung bis zu einem viskoelastischen Gel durchzuführen.

2. Verfahren nach Anspruch 1, wobei das Polysaccharid aus Glucoseaminglucanen ausgewählt ist.

3. Verfahren nach Anspruch 2, wobei das Glucoseaminglucan Hyaluronsäure umfaßt.

4. Verfahren nach einem der vorstehenden Ansprüche, wobei das Vernetzungsmittel aus Epoxiden, Polyaziridylverbindungen und Glycidylethern ausgewählt ist.

5. Verfahren nach Anspruch 4, wobei der Glycidylether 1,4-Butandioldiglycidylether umfaßt.

6. Verfahren nach einem der vorstehenden Ansprüche, wobei das sterische Hindern der Vernetzungsreaktion Verdünnen des wäßrigen Mediums, in dem die Vernetzungsreaktion abläuft, umfaßt, um eine niedrigere Konzentration des Polysaccharids in dem Medium zu erreichen.

7. Verfahren nach einem der vorstehenden Ansprüche, wobei das Wiedereinführen von sterisch ungehinderten Bedingungen Eindampfen des wäßrigen Mediums, in dem die Vernetzungsreaktion abläuft, umfaßt, um eine höhere Konzentration des Polysaccharids in dem Medium zu erreichen.

8. Verfahren nach einem der Ansprüche 1 bis 6, wobei das Wiedereinführen von sterisch ungehinderten Bedingungen Dialysieren des wäßrigen Mediums, in dem die Vernetzungsreaktion abläuft, umfaßt.

9. Verfahren nach einem der vorstehenden Ansprüche, wobei die anfängliche Vernetzungsreaktion in Gegenwart eines polyfunktionellen Vernetzungsmittels bei einem alkalischen pH-Wert abläuft, wodurch Ether vernetzende Reaktionen gefördert werden.

10. Verfahren nach Anspruch 9, wobei der alkalische pH-Wert über pH 9 liegt.

11. Verfahren nach einem der Ansprüche 1 bis 10, wobei die anfängliche Vernetzungsreaktion in Gegenwart eines polyfunktionellen Vernetzungsmittels bei einem sauren pH-Wert abläuft, wodurch Ester vernetzende Reaktionen gefördert werden.

12. Verfahren nach Anspruch 11, wobei der saure pH-Wert bei pH 2 bis 6 liegt.

13. Verfahren nach einem der vorstehenden Ansprüche, wobei die sterische Hinderung der Vernetzungsreaktion durchgeführt wird, bevor das Vernetzungsmittel aufgebraucht worden ist.

14. Verfahren nach einem der vorstehenden Ansprüche, wobei ein biologisch wirksamer Stoff in der vernetzten Polysaccharidgelzusammensetzung während deren Herstellung eingeschlossen wird.

15. Verfahren nach Anspruch 14, wobei der biologisch wirksame Stoff bei Bedingungen mit physiologischem pH-Wert und Salzkonzentration eingeschlossen wird.

16. Verfahren nach einem der Ansprüche 14 und 15, wobei der wirksame Stoff in der Gelzusammensetzung durch Lösen oder Dispergieren derselben in dem aktivierten Polysaccharid, bevor vorstehend erwähntes Polysaccharid in sterisch ungehinderte Bedingungen überführt wird, eingeschlossen wird.

17. Verfahren nach einem der Ansprüche 14 bis 16, wobei der biologisch wirksame Stoff aus Hormonen, Cytokinen, Impfstoffen, Zellen und Gewebe verstärkenden Substanzen ausgewählt ist.

18. Verfahren nach Anspruch 17, wobei die Gewebe verstärkende Substanz ein Polymer, ausgewählt aus Collagen, Stärke, Dextranomer, Polylactid und Copolymeren davon, und Poly-β-hydroxybutyrat und Copolymeren davon, umfaßt.

19. Verfahren nach Anspruch 17, wobei das Hormon aus Erythropoietin und Calcitonin ausgewählt ist.

20. Verfahren nach einem der Ansprüche 14 bis 19, wobei der biologisch wirksame Stoff gegenüber dem Polysaccharid reaktive funktionelle Gruppen enthält und in die Gelstruktur durch chemische Reaktion eingeschlossen ist.

21. Verfahren nach Anspruch 20, wobei der funktionelle Gruppen enthaltende biologisch wirksame Stoff mit einem Vernetzungsmittel für das Polysaccharid vorreagiert worden ist.

22. Verfahren nach Anspruch 21, wobei das Vernetzungsmittel dasselbe Vernetzungsmittel ist, wie es in der Vernetzung des Polysaccharids verwendet wird.

23. Vernetzte biokompatible Polysaccharidgelzusammensetzung, herstellbar durch ein Verfahren nach einem der Ansprüche 1 bis 22.

24. Teilweise vernetzte biokompatible aktivierte Polysaccharidgelzusammensetzung, wie durch ein Verfahren nach

einem der Ansprüche 1 bis 22 erhalten, bevor das Vernetzen des aktivierten Polysaccharids durch Wiedereinführen der sterisch ungehinderten Bedingungen für die Vernetzungsreaktion fortgeführt wird.

25. Zusammensetzung nach Anspruch 23 zur Verwendung als ein medizinisches oder prophylaktisches Mittel.

26. Zusammensetzung nach Anspruch 25, die als ein Depotpräparat eingestellt ist.

27. Verwendung einer Zusammensetzung nach Anspruch 23 für die Herstellung eines medizinischen oder prophylaktischen Mittels zur Gewebeverstärkung eines Säugers, besonders eines Menschen.

28. Verwendung nach Anspruch 27 für die Herstellung eines medizinischen oder prophylaktischen Depotmittels, besonders für die Hormonbehandlung eines Säugers, besonders eines Menschen.

Revendications

1. Procédé pour préparer une composition de gel de polysaccharide biocompatible réticulé, qui comprend :

   la formation d'une solution aqueuse d'un polysaccharide réticulable hydrosoluble ;
   l'initiation d'une réticulation dudit polysaccharide en présence d'un agent de réticulation polyfonctionnel pour celui-ci ;
   l'empêchement stérique de l'achèvement de la réaction de réticulation avant qu'une gélification se produise, un polysaccharide activé étant ainsi obtenu ; et
   la réintroduction de conditions exemptes d'empêchement stérique pour ledit polysaccharide activé de manière à achever sa réticulation jusqu'à un gel viscoélastique.

2. Procédé selon la revendication 1, dans lequel le polysaccharide est choisi dans le groupe consistant en les glucosaminoglucanes.

3. Procédé selon la revendication 2, dans lequel ledit glucosaminoglucane comprend l'acide hyaluronique.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'agent de réticulation est choisi dans le groupe consistant en les époxydes, les composés polyaziridyliques et les glycidyléthers.

5. Procédé selon la revendication 4, dans lequel ledit glycidyléther comprend le diglycidyléther de 1,4-butanediol.

6. Procédé selon l'une quelconque des revendications précédentes, dans lequel ledit empêchement stérique de la réaction de réticulation comprend la dilution du milieu aqueux dans lequel la réaction de réticulation est conduite, pour obtenir une plus faible concentration du polysaccharide dans ledit milieu.

7. Procédé selon l'une quelconque des revendications précédentes, dans lequel ladite réintroduction de conditions exemptes d'empêchement stérique comprend l'évaporation du milieu aqueux dans lequel la réaction de réticulation est conduite, pour obtenir une plus haute concentration du polysaccharide dans ledit milieu.

8. Procédé selon l'une quelconque des revendications 1 à 6, dans lequel ladite réintroduction de conditions exemptes d'empêchement stérique comprend la dialyse du milieu aqueux dans lequel la réaction de réticulation est conduite.

9. Procédé selon l'une quelconque des revendications précédentes, dans lequel la réaction de réticulation initiale en présence d'un agent de réticulation polyfonctionnel est conduite à un pH alcalin, les réactions de réticulation pour la formation d'éthers étant ainsi favorisées.

10. Procédé selon la revendication 9, dans lequel ledit pH alcalin est supérieur à pH 9.

11. Procédé selon l'une quelconque des revendications 1 à 10, dans lequel la réaction de réticulation initiale en présence d'un agent de réticulation polyfonctionnel est conduite à un pH acide, les réactions de réticulation pour la formation d'esters étant ainsi favorisées.

12. Procédé selon la revendication 11, dans lequel ledit pH acide est un pH de 2-6 .

13. Procédé selon l'une quelconque des revendications précédentes, dans lequel ledit empêchement stérique de la réaction de réticulation est accompli avant que ledit agent de réticulation ait été consommé.

14. Procédé selon l'une quelconque des revendications précédentes, dans lequel une substance biologiquement active est incluse dans la composition de gel de polysaccharide réticulé pendant sa préparation.

15. Procédé selon la revendication 14, dans lequel ladite substance biologiquement active est incluse dans des conditions physiologiques de pH et de concentration de sels.

16. Procédé selon l'une quelconque des revendications 14 et 15, dans lequel ladite substance active est incluse dans la composition de gel par dissolution ou dispersion de celle-ci dans ledit polysaccharide activé avant la soummission du polysaccharide mentionné en dernier lieu à des conditions exemptes d'empêchement stérique.

17. Procédé selon l'une quelconque des revendications 14-16, dans lequel ladite substance biologiquement active est choisie dans le groupe consistant en les hormones, les cytokines, les vaccins, les cellules et les substances augmentant les tissus.

18. Procédé selon la revendication 17, dans lequel ladite substance augmentant les tissus comprend un polymère choisi parmi le collagène, l'amidon, le dextranomère, le polylactide et leurs copolymères, et le poly-β-hydroxybutyrate et ses copolymères.

19. Procédé selon la revendication 17, dans lequel ladite hormone est choisie dans le groupe consistant en l'érythropoïétine et la calcitonine.

20. Procédé selon l'une quelconque des revendications 14-19, dans lequel ladite substance biologiquement active contient des groupes fonctionnels réactifs avec le polysaccharide et est incluse dans la structure de gel par réaction chimique avec celle-ci.

21. Procédé selon la revendication 20, dans lequel ladite substance biologiquement active contenant des groupes fonctionnels a été mise à préréagir avec un agent de réticulation pour ledit polysaccharide.

22. Procédé selon la revendication 21, dans lequel ledit agent de réticulation est le même agent de réticulation que celui utilisé dans la réticulation du polysaccharide.

23. Composition de gel de polysaccharide biocompatible réticulé qui peut être préparée par un procédé selon l'une quelconque des revendications 1-22.

24. Composition de gel de polysaccharide activé biocompatible partiellement réticulé tel qu'elle est obtenue par un procédé selon l'une quelconque des revendications 1 à 22 avant la continuation de la réticulation du polysaccharide activé par réintroduction desdites conditions exemptes d'empêchement stérique pour la réaction de réticulation.

25. Composition selon la revendication 23, destinée à être utilisée comme composition médicale ou prophylactique.

26. Composition selon la revendication 25 qui est adaptée sous forme d'une préparation à effet retard.

27. Utilisation d'une composition selon la revendication 23 pour la production d'une composition médicale ou prophylactique pour l'augmentation des tissus d'un mammifère, en particulier d'un être humain.

28. Utilisation selon la revendication 27 pour la production d'une composition à effet retard médicale ou prophylactique, en particulier pour le traitement hormonal d'un mammifère, en particulier d'un être humain.